



## Approach to the Iron deficiency in liver transplant recipients in intensive care

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### Abstract

Preoperative anemia is a common condition in surgical patients, particularly those with end-stage liver failure. Liver transplant (LT) represents the last therapeutic option for end-stage liver failure patients. The procedure is often associated with major blood loss, requiring allogeneic blood product transfusions. The prevalence of anemia in LT recipients ranges from 2% to 28% and the prevalence of iron deficiency (ID) among LT recipients ranges from 45% to 60%. Several factors may contribute to anemia, including occult gastrointestinal bleeding, folate and vitamin B<sub>12</sub> deficiency, autoimmune hemolysis, altered oxide-reductive balance, hypersplenism (in adults), and nutritional deficiency (in children). The intensive care unit (ICU) plays a vital role in the practice of LT recipients. A prolonged ICU stay consumes physical and financial resources. Among LT patients, it may be associated with an increased risk of complications and greater mortality. Preoperative ID may be able to identify patients who are likely to need a prolonged ICU stay after LT because of preoperative ID is associated with high intraoperative PRBC transfusion requirements in LT patients. Furthermore, the quantity of blood products administered intraoperatively is a well known independent risk factor for a prolonged ICU stay after LT. Improvements in preoperative evaluation, surgical techniques, and intraoperative anesthesia of LT recipients during the past decade have resulted in shorter ICU stay. We believe that to avoid prolonged ICU stay, transfusion is important during LT.

**Keywords:** Iron Deficiency; Liver Transplant Recipients; Intensive Care.

More than one-quarter of the world's population is anemic. Approximately one-half of these burdens are a result of iron deficiency anemia. The diagnosis, prevention, and treatment of iron deficiency is clearly a major public health goal. The development of iron deficiency, and the rapidity with which it progresses, is dependent upon the individual's initial iron stores, which are, in turn, dependent upon age, sex, rate of growth, and the balance between iron absorption and loss. The generally lower value for iron stores in adult women, lower caloric intake, use of supplemental iron, and iron losses associated with pregnancy and lactation. Data from the Third National Health and Nutrition Examination Survey (NHANES III; 1988 to 1994) indicated that iron deficiency anemia was present in 1 to 2 percent of adults (1). Iron deficiency without anemia was more common, occurring in up to 11 percent of women (most often premenopausal) and 4 percent of men. In this survey, the prevalence of iron deficiency anemia was significantly higher in older adults, being between 12 and 17 percent in persons 65 years and older (2, 3). The prevalence of anemia in LT recipients ranges from 2% to 28% and the prevalence of iron

deficiency (ID) among LT recipients ranges from 45% to 60% (4,5). Several factors may contribute to anemia, including occult gastrointestinal bleeding, folate and vitamin B<sub>12</sub> deficiency, autoimmune hemolysis, altered oxide-reductive balance, hypersplenism (in adults), and nutritional deficiency (in children).

### Definitions

In most clinical settings, reduced availability of iron is the most important cause of anemia due to impaired erythropoiesis (ie, iron-restricted erythropoiesis). There are two major causes of iron-restricted erythropoiesis:

#### Absolute iron deficiency

In subjects with absolute iron deficiency, iron stores in the bone marrow and other parts of the monocyte-macrophage system in the liver and spleen (previously called the reticuloendothelial system) are absent (ie, absent bone marrow iron stores and/or low serum ferritin levels), making iron unavailable for normal or increased rates of erythropoiesis. This may come about as the result of poor dietary intake of iron, reduced iron absorption, and/or increased blood loss.

#### Functional iron deficiency

Functional iron deficiency is a state in which there is insufficient availability of iron (ie, low plasma iron levels and/or low percent transferrin saturation) for incorporation into erythroid precursors in the face of normal or increased body iron stores (ie, normal to increased bone marrow iron stores and/or normal to increased serum ferritin levels) (6,7).

Received: 28.05.2015

Accepted: 04.12.2015

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### Anemia of inflammation

In the anemia of inflammation (anemia of chronic disease) there is a hepcidin-induced block in the release of iron from the macrophage back into the circulation, making iron less available for red cell production. This is most commonly seen in patients with infection, inflammation, or malignancy. As an example, in a series of 469 patients with a hematologic malignancy, one-third of who were anemic, and a transferrin saturation <20 percent was seen in 35 percent, while a serum ferritin >100 ng/mL was seen in 56 percent (8).

### Causes of Iron Deficiency

#### Blood loss

The major cause of iron deficiency in affluent countries is blood loss, either overt or occult (9). Overt blood loss is, by definition, obvious and not difficult for the clinician to recognize, often by history alone. Examples include severe traumatic hemorrhage, hematemesis, melena, hemoptysis, severe menorrhagia, and gross hematuria. Occult bleeding, on the other hand, may be difficult to track down. This usually occurs via the gastrointestinal tract in men. Although reduced gastrointestinal absorption of iron and a diet deficient in iron can also cause iron deficiency, it is most reasonable to believe, as a first assumption, that iron deficiency reflects blood loss, in order to avoiding missing an occult malignancy or other bleeding intestinal lesion (10).

#### Decreased iron absorption

Gastrointestinal malabsorption of iron is a relatively uncommon cause of iron deficiency, although it may be observed in certain diseases associated with generalized malabsorption or achlorhydria (10). However, the use of proton pump inhibitors, which reduce gastric acid secretion, has not been associated with clinical iron deficiency. Gastrointestinal causes for iron deficiency should be considered in patients with otherwise unexplained iron deficiency, especially when there is refractoriness to oral iron therapy (11).

#### Intravascular hemolysis

Intravascular hemolysis, with its accompanying hemoglobinuria and hemosiderinuria can lead to significant urinary iron losses in patients with paroxysmal nocturnal hemoglobinuria and in cardiac patients with intravascular destruction of red cells secondary to malfunctioning valvular prostheses, patches, or intracardiac myxomas (12)

#### Stages of Iron Deficiency

Normal body iron content: The normal iron content of the body is 3 to 4 grams.

It exists in the following forms: Hemoglobin in circulating red cells: approximately 2 g

Iron containing proteins (eg, myoglobin, cytochromes, catalase) : 400 mg

Plasma iron bound to transferrin: 3 to 7 mg

The remainder is storage iron in the form of ferritin or hemosiderin.

Storage iron in adult men has been estimated as being approximately 10 mg/kg, and is found mostly in liver, spleen, and bone marrow (13). For ferritin levels in the range from 20 to 300 ng/mL, there appears to be a

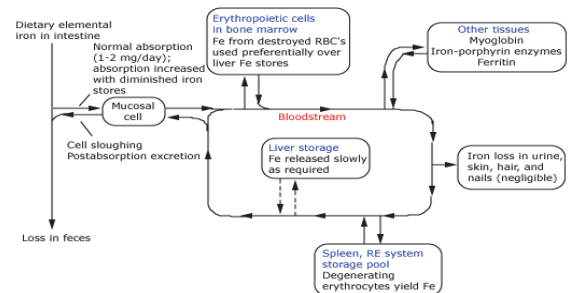
direct quantitative relationship between the ferritin concentration and iron stores (14): Iron stores (mg)  $\approx$  (8 to 10) x ferritin (ng/mL)

#### Progressive iron depletion

The manifestations of iron deficiency occur in several stages (9). These stages are defined by the extent of depletion, first of iron stores and then of iron available for hemoglobin synthesis. Eventually, if negative iron balance continues, the iron and hemoglobin deficiency are so severe that iron deficient red cell production ensues.

In the first stage, iron stores can be totally depleted without causing anemia. The storage iron pool, consisting primarily of ferritin and hemosiderin-bound iron within the monocyte-macrophage system chiefly in bone marrow, liver and spleen, contains approximately 0.8 to 1.0 g of iron in men and about one-half this value in women.

The storage pool can be looked upon as a reserve of iron that can be utilized when there is increased need for hemoglobin synthesis, as in acute blood loss, growth in children and adolescents, pregnancy, lactation, and response to treatment with erythropoietin. Once these stores are depleted, there is still enough iron present in the body within the "labile" iron pool from the daily turnover of red cells for normal hemoglobin synthesis, but the patient is now vulnerable to development of anemia should there be further iron losses (Figure 1).



**Figure 1. Iron homeostasis** Schematic representation of the pathways involved in iron homeostasis. The amount of iron passing from mucosal cells into the body is determined by the rate of erythropoiesis and the state of body iron stores.

Some subjects with extremely low levels of serum ferritin, but without anemia, may have symptoms of fatigue or show decreased exercise tolerance.

Further loss of iron results in anemia, which is initially normocytic with a normal absolute reticulocyte count. This stage of iron deficiency is common in the United States. As an example, it has been estimated that more than 20 percent of menstruant women in the United States have no iron reserves and are in this stage (12). Common laboratory findings at this stage include: Low levels of ferritin and serum iron (Fe) Increased levels of transferrin (Tf, total iron binding capacity, TIBC)

Low percent saturation of transferrin (ie, Fe/TIBC or Fe/Tf, stated as a percent) Increased unsaturated iron binding capacity (UIBC = TIBC - Fe)

More profound deficiency results in the classical hypochromia (low mean corpuscular hemoglobin) and microcytosis (low mean corpuscular volume) of iron-deficient erythropoiesis.

### Changes in iron metabolism

In uncomplicated iron deficiency anemia, both the anemia per se and the absent iron stores provide a message to stop production of hepcidin, a protein produced in the liver that is thought to be a major regulator of iron balance. The absence of hepcidin enhances gastrointestinal iron absorption as well as the release of iron from stores in macrophages. This subject is discussed in detail separately. Hemoglobin was low at 8 g/dL; mean cell volume (MCV) was low at 75 fL.

The serum iron was low (10 mcg/dL) and the total iron binding capacity (TIBC, transferrin) was elevated (400 mcg/dL), resulting in a low transferrin saturation of 2.5 percent. The plasma ferritin concentration was markedly reduced (10 ng/mL).

Iron stores were absent in the patient's bone marrow as judged by microscopic examination of the Prussian Blue reaction on a bone marrow aspirate, the "gold standard" test for estimating iron stores.

Finally, the patient responded briskly to a therapeutic trial of oral iron, with a reticulocytosis, followed by elevations in the hemoglobin concentration and hematocrit.

The current reality in developed countries is that this classic presentation is uncommon, and that the diagnosis and management of iron deficiency anemia is usually more complicated. As an example, many, if not most, patients with iron deficiency anemia in the United States have normal red cell indices and a relatively normal peripheral blood smear. In older adults, iron deficiency anemia may have an insidious onset and present with symptoms related to exacerbation of an underlying comorbidity (eg, increasing angina from coronary artery disease, increased confusion in subjects with dementia, increased dyspnea in those with congestive failure).

### Serum or plasma ferritin

The serum or plasma ferritin concentration is an excellent indicator of iron stores in otherwise healthy adults and has replaced assessment of bone marrow iron stores as the gold standard for the diagnosis of iron deficiency in most patients (Figure 2) (15). The ferritin concentration ranges from 40 to 200 ng/mL (mcg/L) in normal subjects, and is markedly elevated in states of iron overload, due to stimulation of hepatic ferritin synthesis and release by iron (16).

### Inflammatory states

Ferritin is an acute phase reactant, with plasma levels increasing in liver disease, infection, inflammation, and malignancy. As an example, the synthesis and release of ferritin by hepatic cells is directly enhanced by inflammatory cytokines such as interleukin-1 and tumor necrosis factor (16). Thus, a patient with iron deficiency along with a component of inflammation may have a "falsely" normal ferritin concentration. Examples include

subjects with rheumatoid arthritis, the anemia of (chronic) inflammation, and the anemia associated with heart failure.

The effect of inflammation is to elevate serum ferritin approximately threefold (14). A useful rule-of-thumb in such patients is to divide the patient's serum ferritin concentration by three; a resulting value of 20 or less (ie, an initial serum ferritin <60 ng/mL) suggests concomitant iron deficiency. Although high plasma transferrin and low mean cell volume showed similar predictive values, more patients with iron deficiency anemia could be diagnosed by serum ferritin measurements than by other conventional blood tests.

### Serum iron and transferrin (TIBC)

In iron deficiency anemia, the serum iron concentration (SI) is reduced, and the level of transferrin (also measured as total iron binding capacity [TIBC]) is elevated; the latter finding reflects the reciprocal relationship between serum iron and transferrin gene expression in most nonerythroid cells (17). The low SI and high transferrin/TIBC result in a low transferrin saturation or index (saturation = SI/TIBC x 100), often to levels less than 10 percent, compared with the normal values of 25 to 45 percent (18).

### Bone marrow iron

Iron in bone marrow macrophages and erythroid precursors (sideroblasts) can be detected with the Prussian Blue stain on marrow spicules. Lack of stainable iron in erythroid precursors as well as marrow macrophages is considered by most clinicians to be the "gold standard" for the diagnosis of iron deficiency. In contrast, in uncomplicated anemia of chronic disease, iron is present in marrow macrophages but absent or reduced in erythroid precursors.

However, bone marrow sampling and testing for stainable iron is expensive, invasive, and usually unnecessary (15). It has been replaced in practice by measurement of serum ferritin.

### Serum transferrin receptor

Circulating transferrin receptor (sTfR) is derived from bone marrow erythroid precursors. It provides a quantitative measure of total erythropoietic activity, since its concentration in serum is directly proportional to erythropoietic rate and inversely proportional to tissue iron availability (17). Thus, iron deficient patients generally have increased levels of sTfR.

### TfR-ferritin index

Calculation of the ratio of sTfR (expressed as mg/L) to ferritin (expressed as mcg/L), or the ratio of sTfR to the logarithm (to the base 10) of the ferritin concentration, may overcome some, but not all (19), of the above-noted limitations of measurement of sTfR, and may be especially useful for estimating total body storage of iron in epidemiologic studies (20), and for distinguishing between iron deficiency anemia (IDA) and the anemia of chronic disease (ACD, anemia of chronic inflammation) (21).

This ratio presumably is effective in making this distinction since the numerator (sTfR) is increased in IDA

and normal in ACD, while the denominator (ferritin or log ferritin) is decreased in IDA and normal to increased in ACD. Specifically, a sTfR/log ferritin ratio (TfR-ferritin index)  $<1$  suggests the diagnosis of the anemia of chronic disease (ACD), while a ratio  $>2$  suggests the presence of iron deficiency anemia (IDA) (Weiss, 2005). Patients with the combination of IDA and ACD will also have a TfR-ferritin index  $>2$ .

### Red cell morphology and indices

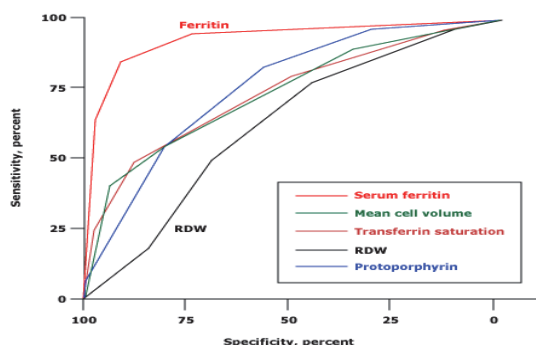
Despite the classic description of iron deficiency as leading to a hypochromic, microcytic anemia, many iron deficient patients in Western countries will have normal red cell morphology. Further, the finding of a hypochromic microcytic anemia is not pathognomonic of iron deficiency with thalassemia and, less commonly, the anemia of chronic inflammation being the other common conditions encountered in daily practice. It is important to rule out these disorders before beginning a trial of iron therapy, since many patients with thalassemia or chronic inflammation are already iron overloaded.

### Reticulocyte indices

With the advent of automated counting of reticulocytes, several reticulocyte parameters are available to clinicians and pathologists (22). These include reticulocyte volume, reticulocyte hemoglobin content, and reticulocyte hemoglobin concentration.

### Red cell zinc protoporphyrin level

The last step in the biosynthesis of heme is the addition of iron to protoporphyrin IX. If iron is unavailable, zinc (Zn) substitutes, forming zinc protoporphyrin (ZPP), which can be measured. This test measures the lack of iron, not why it is unavailable. Thus, the red cell ZPP level will be increased in both iron deficiency and the anemia of chronic inflammation in which iron is present but trapped in macrophages and not "available" for heme synthesis. The sensitivity and specificity of an increased ZPP for iron deficiency in adults are significantly less than that of serum ferritin (Figure 2) (23).



**Figure 2. Testing for iron deficiency** Receiver operating characteristic (ROC) curves showing the sensitivity and specificity of various tests for the diagnosis of iron deficiency [ferritin, red cell protoporphyrin, transferrin saturation, mean red cell volume (MCV), and red cell volume distribution width (RDW)]. Note that, at any given level of sensitivity or specificity, serum ferritin outperforms all the other tests shown.

### Iron deficiency Anemia in Liver Transplant Patients Characteristics

Anemia is a common problem in the intensive care unit (ICU), occurring frequently in critically ill patients. Observational studies indicate an incidence of approximately 95% in patients who have been in the ICU for 3 or more days (24). The presence of anemia has been associated with worse outcomes including increased lengths of stay and increased mortality. The etiology of anemia is multifactorial and includes: It is being increasingly recognized that the anemia in a majority of critically ill patients is a result of poor iron utilization akin to that seen in the so-called anemia of chronic disease. Decreased serum iron, serum transferrin, transferrin saturation, and increased ferritin levels have been found in these patients, indicative of an inflammatory profile. In one study of 51 postoperative critically ill patients, decreased serum iron and increased ferritin were found in more than 75% of patients (25). Forty-one of these patients suffered 56 septic episodes. These were accompanied by a marked decrease in serum transferrin and increase in serum ferritin accompanied by a reduction in hemoglobin. Recovery from sepsis was accompanied by a significant improvement in hemoglobin, serum iron, and transferrin.

### Clinical Finding

Key symptoms of anemia, such as dyspnea and tachycardia, are caused by decreased blood oxygen levels and peripheral hypoxia. Compensatory blood shifting from the mesenteric arteries may worsen perfusion of the intestinal mucosa. Motility disorder, nausea, anorexia, and even malabsorption have been attributed to anemia. Reduced metabolic and energy efficiency during physical activity also contribute to weight loss in anemia. Central hypoxia may lead to symptoms such as headache, dizziness, vertigo, or tinnitus. Several studies have confirmed that treatment of anemia improves cognitive function. Iron is a component of hemoglobin myoglobin, cytochromes, and many other enzymes (26). Since the majority of iron in the body is used for synthesis of hemoglobin, the most important finding of ID is anemia. In ID anemia, clinical findings secondary to anemia may be found as in all anemias or the diagnosis can be made during laboratory investigations in the absence of any clinical finding. Preoperative anemia is a common condition in surgical patients, especially in end-stage liver failure due to anemia of LT candidates. ID anemia can be related to cirrhosis, hypersplenism, renal insufficiency in liver transplant patients. Iron deficiency plays a major role in persistent anemia in the immediate posttransplant period.

### Distinctive features

The high prevalence of anemia among cirrhotic patients may have several reasons. First, most LT recipients usually suffer from cirrhosis-associated chronic liver disease. Iron stores may be rapidly depleted posttransplant due to surgical blood loss, frequent phlebotomy, and utilization of stores for enhanced erythropoiesis. ID diagnosis using these criteria may be confounded by the presence of inflammation due to elevation of ferritin, an acute-phase response protein in



OLT patients (27). However, the accurate assessment of iron deficiency posttransplant is limited by available assays. Ferritin is a well-known acute-phase reactant, and elevated levels may represent superimposed illness (eg, acute rejection and infection). Ferritin levels drop with iron utilization, but also rise with increased gastrointestinal iron absorption following transplantation. Additionally, advanced liver disease can contribute to low CRP levels because of reduced hepatic protein synthesis (28). Also, high ferritin and transferrin saturation may mask some of those with chronic hepatic C infection liver disease who might be normal or low in iron. Therefore, the biochemical parameters we used for diagnosis not only are markers for ID but also can indicate activation of acute-phase responses. Patients with evidence of iron homeostasis were further evaluated using peripheral blood smears to correlate biochemical data.

### Treatment Options in ICU

The ICU plays a vital role in the practice of LT. A prolonged ICU stay consumes physical and financial resources. Among LT patients, it may be associated with an increased risk of complications and greater mortality (29). The main principles in treatment of iron deficiency anemia include making the diagnosis, investigating the condition which causes iron deficiency and elimination of this condition, replacement of deficiency, improvement of nutrition and education of patients and families. The rationale for treatment of ID anemia in ICU is twofold: (1) to improve oxygen delivery to tissues and (2) because of the poorer prognosis associated with its presence.

### Red Cell Transfusions

Target hemoglobin of 10 g/dL has been accepted as a minimum threshold to transfuse since its first proposal in 1942: the 10/30 rule (30). Oberkofler et al. showed that >10 units of FFP and > 7 units of RBC transfused intraoperatively were independent risk factors for a prolonged ICU stay (34). This practice remained unchanged for decades despite the lack of evidence to support its use. With increasing recognition of risks associated with blood transfusions such as transmission of infections, TRALI, allergic reactions, fluid overload, and immunomodulation, there has been a more critical appraisal of the need of blood transfusions in this population of patients (31). The transfusion rate during the ICU stay was 37%. Mortality rates were significantly higher in patients who were transfused versus those who were not. There was a dose-response effect with a higher mortality seen in patients receiving increased number of transfusions. A multicenter, randomized, controlled trial comparing restricted (transfusion for hemoglobin <7 g/dL to maintain target hemoglobin of 7–9.0 g/dL) with liberal transfusions (transfusion for hemoglobin <10 g/dL to maintain target hemoglobin 10–12 g/dL) in critically ill patients carried out by the Transfusion Requirements in Critical Care Investigations (TRICC) showed that a restrictive strategy is at least as good as the more liberal one and significantly better in patients who were less acutely ill and younger than 55 years (32). Current recommendations advocate the use of restricted red blood cell transfusion practice, limiting

transfusions to those clinically indicated rather than transfusing to an “ideal” target hematocrit (33).

### Iron Therapy

The recognition of a significant subgroup of critically ill patients with both ID suggests a possible role for iron therapy. Patients scheduled for elective surgery must be monitored 3-4 weeks preoperatively (34). After starting iron therapy, an increase in reticulocyte count occurs within 2 week, hemoglobin rises by 2 g/dL within 4 week, and hemoglobin level returns to normal within 8 week. To assess the response to therapy, hemoglobin should be measured within 4 week of the initiation of iron therapy. After 4 week of iron therapy, response to treatment is considered appropriate or optimal, if hemoglobin rises by at least 2 g/dL; partial, if hemoglobin rises by 1-1.9 g/dL; absent, if hemoglobin rises less than 1 g/Dl (35). The difficulty lies in accurate diagnosis because the tests with the best discriminatory power such as sTfR/log ferritin ratios and hepcidin levels are not commercially available and have not been clinically validated. It also must be kept in mind that iron can cause oxidative stress and theoretically increase the risk of bacterial infection. However, observational studies have not shown any association between iron administration and risk for infection. An algorithm has been suggested use of C-reactive protein (CRP), serum ferritin, sTfR/log ferritin ratios, and hepcidin levels to make a decision regarding use of iron. Patients are considered to have both ID if they have reduced hepcidin levels and reduced sTfR/log ferritin ratios in the face of elevated CRP and ferritin levels. These patients would likely benefit from judicious administration of iron (36).

### Conclusion

Preoperative ID may be able to identify patients who are likely to need a prolonged ICU stay after LT because of preoperative ID is associated with high intraoperative PRBC transfusion requirements in LT patients. Improvements in preoperative evaluation, surgical techniques, and intraoperative anesthesia of LT recipients during the past decade have resulted in shorter ICU stay. We believe that to avoid prolonged ICU stay, transfusion is important during LT.

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